SYNOPSIS

FOR

Ph.D. REGISTRATION

"Studies on effects of aqueous extract of *Achyranthes aspera* plant in regulation of thyroid function in *Albino rats*"

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STATE OF ART:

Thyroid problems are among the most common endocrine disorders presently seen worldwide[1]. About 1 to 2 % of the adult population is known to suffer from thyroid disorders[2]**.** According to the world Health Assembly report, about 1.5 billion persons in more than 110 countries are threatened with thyroid disorders[1]**.**

Hypothyroidism generally describes an under-active thyroid that does not produce enough thyroid hormones causing an overall decrease in physical and mental activity[1]**.**The causes of hypothyroidism includes:-Thyroid gland failure to produce hormones (T_3 , T_4), deficiency of TRH,TSH or both and inadequate supply of dietary iodine.

Recently, the importance of the traditional indigenous medicines has been realized because of the side-effects of the modern medicines, failure to treat deadly diseases, costly treatment and unavailability of medical facilities in remote places .The WHO has also recognized the importance of herbal medicines and stated that 80% of the population in developing nations is still dependent on herbal therapy for remedy of diseases. Medicinal plants as a whole are either known to be of therapeutic values or their parts(root ,stem ,leaves ,flowers, seeds and fruits) show medicinal properties[3]**.**

Ayurvedic or natural herbal medicine is common in Jharkhand traditional medicinal system by tribal people because major part of tribal population in Jharkhand are under low economical status, living in remote areas where advanced and modern medicinal facilities are not available . In order to provide cheap and affordable curative agents against diseases, it is suggested that scientific attention be given to the traditional use of the natural medicinal plants for therapeutic purpose, the scientific validation of natural medicinal plants will lead to improvement of health in people of Jharkhand.

PROBLEMS TO BE INVESTIGATED:

In present experiment attempts will be made to investigate the potentiality of leaf, stem and root extracts of *Achyranthes aspera* as a useful agent for the regulation of thyroid metabolism.

OBJECTIVE OF THE INVESTIGATION:

- Efficacy of *Achyranthes aspera* leaf, stem and root will be established in regulation of thyroid function in *Albino rat*.
- To study the haematological effect of *Achyranthes aspera* in experimental *Albino rat*.
- Histopathological studies of thyroid gland in different experimental conditions will be studied.
- To investigate the phytochemical properties of the leaf , stem and root extracts separately.

MOTIVATION:

- *Achyranthes aspera* is used by the ethnic communities in their traditional system of medicines for remedy of different types of diseases such as diabetes, piles, rheumatism, cancer, renal disorder and many more[3].
- *Achyranthes aspera* is abundantly found in Jharkhand. It is an annual erect woody base weed found in road sides. So, it will be easily available for experimental work.
- Clinically, it has been observed that since levothyroxine replacement therapy requires lifelong treatment, it is associated with poor compliance in some patients[4].

So, it is very important to show an interest in indigenous system of medicine and traditional herbal remedies which are regarded as quite safe with no side effects and are cost effective.

HYPOTHESIS:

The current mode of treatment for hypothyroidism is levothyroxin replacement therapy[5]. Recent studies have reported that a significant number of hypothyroid patients on levothyroxine replacement therapy experience decreased neurocognitive function and lead poorer quality of life despite being biochemically euthyroid[6,7] . Major parts of tribal population in Jharkhand are under low economical status, living in remote areas where advanced and modern medicinal facilities are not available. So, attention must be given to the traditional use of natural medicinal plants which will lead to the improvement of health in peoples of Jharkhand.

RESEARCH DESIGN AND METHODOLOGY:

Plant collection

The plant *Achyranthes aspera* (common name:chaff-flower,prickly chaff flower,devil's horsewhip) will be collected from the Lalpure locality of the city Ranchi (Jharkhand).

Plant extract preparation

Freshly collected plant will be thoroughly washed with tap water followed by distilled water .Then the plant sample will be shade dried inside the hot air oven at 50℃ and then leaf ,stem and root sample will be powdered separately using mechanical grinder[8].

Preparation of aqueous extract of *Achyranthes aspera* leaf, stem and root separately

125 g of the powdered leaf , stem and root extract of *Achyranthes aspera* will be weighed separately and macerated in 1000 ml of deionised distilled water for 12 h at room temperature and filtered with nylon cloth[9] . The filtrats of leaf,stem and root of *Achyranthes aspera* will be then evaporated to dryness in an oven (ewerka oven) at 40· C .

Experimental Animals

24 adult *Albino rats* of the same age (6 weeks) and weight of about 120-150 g will be used in the study. Animals will be maintained under standard condition of ventilation , temperature (25± 2˚ c) humidity (60-70 %) and light/dark condition (12/12 h). Rats will be housed in stainless steel cages and will be provided with free access of food and drinking water *ad libitum* . After two weeks of acclimatization animals will be divided into 6 groups (n=3 each)**[10].**

Hypothyroidism by inducing methimazole

Hypothyroidism will be induced by daily oral administration of 25 mg/kg methimazol for five successive days**[11].**

Dose of aqueous extract of *Achyranthes aspera* leaf, stem and root.

The rats will be treated with aqueous extract of *Achyranthes aspera* leaf, stem and root 400 mg/kg for a period of 14 and 28 days after hypothyroidism[12].

Experimental Design

Initial body weight will be taken of each experimental rats . Blood samples will be collected from control and all experimental animals by cardiac puncture under anesthetic condition for estimation of blood parameters in beginning of experiment by overnight fasting.

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G I – (CONTROL GROUP) ;- Control group animals will be provided with usual rat feed and water for 33 days.

GII- (HYPOTHYROID GROUP);- Rats will be orally administered with methimazole (25 mg/kg bwt.) for five successive days.

GIII –(LEAF TREATED GROUP FOR 14 DAYS) ;Hypothyroidism will be induced in animals of group III with methimazole (25 mg/kg bwt.) for five successive days and then they will be treated with aqueous extract of *Achyranthes aspera* leaf (400 mg/kg bwt.) for a period of 14 days. After 14 days of treatment blood will be collected through cardiac puncture after anaesthetizing with ether and thyroid glands were collected after sacrificing the animals.

G IV –(LEAF TREATED GROUP FOR 28 DAYS) ;- Animals of group IV will be induced hypothyroidism with methimazole (25 mg/kg bwt.) for five successive days and then they will be treated with aqueous extract of *Achyranthes aspera* leaf (400 mg/kg bwt.) for a period of 28 days. After 28 days of treatment blood will be collected through cardiac puncture. Animals were sacrificed under anaesthesia and thyroid glands will be collected and stored for further analysis.

GV–(STEM TREATED GROUP FOR 14 DAYS) ;Hypothyroidism will be induced in animals of group V with methimazole (25 mg/kg bwt.) for five successive days and then they will be treated with aqueous extract of *Achyranthes aspera* stem (400 mg/kg bwt.) for a period of 14 days. After 14 days of treatment blood will be collected through cardiac puncture after anaesthetizing with ether and thyroid glands will be collected after sacrificing the animals.

GVI –(STEM TREATED GROUP FOR 28 DAYS) ;- Animals of group VI will be induced hypothyroidism with methimazole (25 mg/kg bwt.) for five successive days and then they will be treated with aqueous extract of *Achyranthes aspera* stem (400 mg/kg bwt.) for a period of 28 days. After 28 days of treatment blood will be collected through cardiac puncture. Animals will be sacrificed under anaesthesia and thyroid glands will be collected and stored for further analysis.

GVII –(ROOT TREATED GROUP FOR 14 DAYS) ;Hypothyroidism will be induced in animals of group VII with methimazole (25 mg/kg bwt.) for five successive days and then they will be treated with aqueous extract of *Achyranthes aspera* root (400 mg/kg bwt.) for a period of 14 days. After 14 days of treatment blood will be collected through cardiac puncture after

anaesthetizing with ether and thyroid glands will be collected after sacrificing the animals.

GVIII –(ROOT TREATED GROUP FOR 28 DAYS) ;- Animals of group VIII will be induced hypothyroidism with methimazole (25 mg/kg bwt.) for five successive days and then they will be treated with aqueous extract of *Achyranthes aspera* root (400 mg/kg bwt.) for a period of 28 days. After 28 days of treatment blood will be collected through cardiac puncture. Animals will be sacrificed under anaesthesia and thyroid glands will be collected and stored for further analysis

Blood sample will be collected by cardiac puncture method under anesthetic condition on 5 th,14th and 28 th day after overnight fasting.

PHARMACOLOGICAL PARAMETERS

(A) Estimation of serum thyroid profile in control & different experimental groups.

For estimation of serum thyroid profile in control and different experimental groups.Serum level for T_3, T_4 and TSH will be estimated by using Chemiluminescence Immunoassay Analyzer (C.L.I.A)[13]

(B) Estimation of serum lipid profile in control & different experimental groups.

For estimation of lipid profile hormone levels in control and different experimental groups.Serum levels for total cholesterol (TC),triglyceride (TG),low density lipoprotein(LDL),high density lipoprotein (HDL) will be performed by spectrophotometry[14].

HISTOLOGICAL TECHNIQUES

The thyroid gland will be rapidly removed and fixed overnight in the Bouin's fixative, then the thyroid gland will be dehydrated with grades of ethanol (70, 90 and 100%). Dehydration will be then followed by clearing the samples in 2 changes of xylene. Samples will be then impregnated with 2 changes of molten paraffin wax, then embedded and blocked out. Paraffin sections (5-6 μm) thick transverse sections of the thyroid glands will be cut using a rotary microtome and mounted on glass slides. The slides will be stained with haematoxylin and eosin (H&E) according to Bancroft and Gamble, (2008)[15]. Stained sections of control and treated rats will be examined under the light microscope (Olympus CH Japan) for alterations in the architecture of the thyroid gland especially the thyroid follicular cells,epithelial cells,presence of colloid,changes in height of epithelial cell,Thyroid follicle numbers of small follicles,average follicles and large follicles. All experiments will be conducted in accordance with the guidelines approved by the Ranchi University ,Ethical Committee.

PHYTOCHEMICAL ANALYSIS

The qualitative analysis of secondary metabolites will be carry out by the following standard methods[16].

(1) Test for Alkaloids

. Mayer's test.

(2) Test for Flavonoids

. Alkaline reagent Test

(3)Test for Tannins and Phenolic Compounds

Ferric chloride test:.

.(4)Test for Cardio Glycosides

Keller-Killani Test

(5)Test for Saponins

Frothing Test:.

(6)Test for Terpenoids

Liebermann test**:**.

STATISTICAL ANALYSIS

The data were analyzed as meas ± S.D statistical analysis were carried out by using student's "t" test.

INSTRUMENTS TO BE USED

- Weighing machine
- What man's filter paper
- Metal cages
- Oral gauge
- Microtome
- Refrigerator
- Beaker
- Measuring cylinder
- mechanical grinder

CHEMICALS

- Iodine (solution)
- Pot. Iodide (powder)
- Distilled water
- Glacial acetic acid
- Conc. Sulphuric acid
- Ferric chloride
- Sodium hydroxide
- Ethanol

SIGNIFICANCE OF THE STUDY:

Medicinal plants have a great role in providing health and treatment as well as disease prevention in human communities. Medicinal herbs are very rich in secondary metabolites which have profound physiological effects on the function of mammalian tissues in health and disease conditions.

Traditional knowledge of herbal drugs has been inherited from generation to generation by words of mouth and preserved with practice only. Tribal healer's use herbal drugs for remedy of different diseases as a traditional system of medicines . In India the reference of the curative properties of some herbs in Rig Veda seems to be the earliest record of the use of plants in medicine[3].

RELEVANCE TO THE PRESENT DAY PROBLEM:

The current mode of treatment for hypothyroidism is levothyroxine replacement therapy. However, there are certain limitations associated with levothyroxine replacement therapy . Recent studies have reported that a significant number of hypothyroid patients on levothyroxine replacement therapy experiences decreased neurocognitive function, lifelong treatment and lead poorer quality of life despites being biochemically euthyroid[6,7].

Hence, there is an urgent need for more effective therapeutic strategies to treat hypothyroidism .It is very important to show an interest in indigenous system of medicine and traditional herbal remedies which are regarded as quite safe with no side effects and are cost effective.

NEED OF THE SOCIETY:

Recently , the importance of the traditional indigenous medicines has been realized because of the side-effects of the modern medicines , failure to treat deadly diseases, costly treatment and unavailability of medical facilities in remote places[3].Ayurvedic or natural herbal medicine is common in Jharkhand traditional systems by tribal people because major part of tribal population in Jharkhand are under low economical status , living in remote areas where advanced and modern medicinal facilities are not available . In order to provide cheap and affordable curative agents against diseases, traditional use of the natural medicinal plants for therapeutic purpose should be encouraged.

CONTRIBUTION TO THE EXISTING KNOWLEDGE:

Achyranthes aspera is a species of plant in the Amaranthaceae family , commonly known as apmarga or chirchita in Hindi. It is a popular medicinal plant which has occupied a pivotal position in folk medicine. It is distributed throughout the tropical world and it is commonly found as a weed throughout India. Pharmacological and medicinal uses of *Achyranthes aspera* has been studied at Amity university[17]. Antioxidant and antibacterial activity of *Achyranthes aspera* plant extract in different organic solvent has been studied at Auxilium college,Velleore Tamil Nadu[18]. Antidiabetic activity of *Achyranthes aspera* has been studied at Standard Firework Rajaratnam college for women , Sivakasi, India[19]. *Achyranthes aspera* leaf extract inhibited fungal growth has been studied at Voorhees college, Vellore ,India[20]. In present work, attempts will be made to investigate the potentiality of leaf , stem and root aqueous extracts of *Achyranthes aspera* as a useful agent for the regulation of thyroid metabolism.

FUTURE WORK:

Exploration of the chemical constituents of the plants and pharmacological screening may provide us the basic need for development of novel agents . In addition , natural herbs have provided us some of the very important life saving drugs used in modern medicine.

However , among the estimated 250,000 – 400,000 plant species, only 6% have been studied for biological activity and about 15% have been in vestigated phytochemically (cragg et al , 1997). This shows a need for planned activity , that leads the phytochemical evaluation of natural herbal drugs.

REFERENCES:

1. Tarun sharma[1], Malvika[2],Monu Gupta[3],Sumit Nathani[4].Review of ayurvedic drugs acting on hypothyroidism. *International Ayurvedic Medical Journal* ISSN:2320 5091.
2. Pocock , G. and Richards(1999), CD., The thyroid gland, in *Human Physiology: The Basis of Medicine*, Oxford University Press, New York, p. 212.
3. Narendra kumar pandey*,Sharma H.P.,Patnaik Amit , Jain Paras(2013) . '' A review on potential magic folk herbal medicinal plant: *Achyranthes aspera* L. International Journal of Medicinal Plants. Photon 105 , 350-363.
4. Chakera AJ , Pearce SH, Vaidya B(2012). Treatment for primary hypothyroidism: Current approaches and future possibilities. Drug Des Devel Ther. ;6:I—ll.
5. S.Ashwini,Zachariah Bobby ,M.G. Sridhar, and C.C. Cleetus (2017 Jan-Mar). Insulin Plant (*Costus pictus*) Extract Restores Thyroid Hormone Levels in Experimental Hypothyroidism. Pharmacognosy Res.: 9(1): 51-59.
6. Saravanan P, Chau WF, Poberts N,Vedhara K, Dayan CM(2002). Psychological well-being in patients on `adequate' doses of l-thyroxine: Results of a large, controlled community-based questionnaire study.Clin Endocrinol (Oxf) ;577-85.
7. Wekking EM, Appelhof BC, Fliers E, Schene AH, Huyser J ,Tijssen JG, et al(2005). Cognitive functioning and well-being in euthyroid patients on thyroxine replacement therapy for primary hypothyroidism. Eur J Endocrinol. ;153:747-53.
8. Binit Baraik, Paras Jain* and H.P. Sharma(2014). *Achyranthes aspera* L.:As a Source of Bio-fungicide. American Journal of Advanced Drug Delivery. ISSN 2321-547X.
9. Kamtchoving P, Mbongue Fndio G, Dimo T, Jatsa II(2002). Evaluation of androgenic activity of *Zingiber officinale and Pentadiplandra brazzeana* in

maie rats. Asian J Androl ; 4: 299-301.

10. Saleh, Eman M(2012). Antioxidant effect of aqueous extract of propolis on hepatotoxicity induced by octylphenol in male rats. Acta Toxicol. Argent. 20 (2): 68-81.
11. Francavilla, S; Cordeschi , Properzi, G; Di cicco, L; B and D' ARMIENTO. Effect of thyroid hormone on the pre- and post-natal development of the rat testis. J. Endocrinol., 129: 35-42.
12. Fatema Zohura Talukder,Kousik Ahmed Khan,Riaz Uddin, Nusrat Jahan,Md Ashraful Alam (2012).In vitro free radical scavenging and anti-hyperglycemic activities of *Achyranthes aspera* extract in alloxan-induced diabetic mice.Department of pharmacy ,Stamford University Bangladesh,Dhaka,Bangladesh;Drug Discoveries & Therapeutics.2012;6(6):298-305
13. Venkatamani Yaamarthy,C Jhansi Rani,R Hemamalini,Mohd Iqbal Ahmed(2023).Evaluation of serum cortisol levels in patients with hypothyroidism at a tertiary care hospital,Telangana,India:A case-control study.Journal of clinical and Diagnostic Research.2023 mar,Vol-17(3):BC05-BCO7.
14. Kanwalpreet Kaur,Karuna Thapar,Preeti Malhotra(2020).Cord lipid profile comparison of newborns of hypertensive mothers.Department of pediatrice,Sri Guru Ram Das Institute of Medical Sciences AND Research,Amritsar,Punjab,India.International Journal of Contemporary paediatrics,2020 may;7(5): 1038-1042.
15. Bancroft,J.D,Gamble,M.,(2008).Theory and practice of histological techniques,Elsevier health sciences.
16. Tripathi Keerti,Rai O.P,Nigam .R.S(2023).DEPARTMENT OF CHEMISTRY,Govt.P.G. college,Satna (M.P).International Journal of Innovative Science and Research Technology.Vol-8,December-2023,ISSN No-2456-2165.

17. Saba Hasan (2014), Pharmacological and medicinal uses of *Achyranthes aspera*.Amity Institute of Biotechnology, Amity University , Lucknow (U.P). International Journal of Science,Environment and Technology, Vol. 3,No 1,123-129.
18. Abi Beaulah G[1], Mohammed Sadiq A[2] and Jaya Santhi R[1]*(2011). Antioxidant and antibacterial activity of *Achyranthes aspera*: An in *vitro* study.Department of Chemistry, Auxilium college,Vellore, Tamil Nadu, India. Annals Biological Research, 2(5):662-670.
19. Geetha K(2016). Antidiabetic activity of *Achyranthes aspera* L. with alloxanised mice for the estimation of level of glucoses and cholesterol. Department of Botany,The Standard Fireworks Rajaratnam college for women,Sivakasi,India. Asian Journal of Plant Science and Research, 6(2):18-23.
20. Ek.Elumalai[1],N.chandrasekaran[2],T.thirumalai[1], C.sivakumar[1] S.viviyan therasa[1],E david[1]*(2009). *Achyranthes aspera* leaf extracts inhibited fungal growth. [1]P.G and Research Department of Zoology, Physiology wings, Voorhees college,Vellore-632001(T.N) India. International Journal of PharmTech Research, Vol.1,No.4,pp 1576-1579.